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Contents

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Density growing : a method for local improvement of electron density maps

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1. Introduction. Dummy atoms and phase improvement.

The procedure (Watenpaugh et al. (1973)) of refinement of an atomic model in reciprocal space and phase recalculation from the refined model has been proven as a powerful tool to correct model errors and to build their missing parts. However, in general, the density corresponding to the missing atoms is twice weaker than the one for the atoms included in the phase calculation (Raman, 1959; Main, 1979). Since not-yet-interpreted regions of the map usually correspond to less ordered parts of the molecule and the density in these regions is already weak, the problems of density improvement for such regions is quite acute.

Agarwal and Isaacs (1977) suggested to improve the density by placing dummy atoms in the unit cell with a following «refinement» of such model. Dummy atoms are placed respecting the known interatomic distances in the peaks of the initial map. The phases calculated from the refined dummy-atoms model are used together with the observed amplitudes to obtain new, improved maps.

This idea was further developed by Lunin & Urzhumtsev (1984, 1985) by suggesting new schemes for model building and model modification and by the development of the likelihood approach for phase error estimation in order to calculate properly weighted improved syntheses. Their mixed models contain both «molecular atoms» from the interpreted parts of the map and dummy atoms for uninterpreted ones. Dummy atoms interact with the molecular atoms, however they are really artificial in the sense that they can be at any distance to each other, which is essential in order to improve the map details.

The method was successfully applied in several cases at middle resolution (2.3-3.5Å). A

development of this approach for the high-resolution case (Lamzin & Wilson, 1993) resulted in the package ARP distributed through CCP4 (CCP4, 1994). *[Actually, ARP is not at present part of the CCP4 suite -Ed.]*

However, even with all these modifications, the method had three major problems :

a) incorrect phase error-estimations for the refined models;
b) impossibility to retrieve missing density;
c) impossibility of phase extension starting from a resolution lower than approximately 3.5 Å.

The first problem has been recently solved by Lunin & Skovoroda (1995). To solve the second problem, Agard and co-authors (Wilson & Agard, 1993; Baker et al., 1993; Bystroff et al., 1993) used simultaneously the properties of atomicity and connectivity. Their new approach realised through the program PRISM (Wilson & Agard, 1993) allows to build «bridges» between regions with reasonably strong density and to recover missing density in the middle. However, the presence of some initial peaks of density near the poorly defined regions is necessary.

2. Density growing : method description.

The initial idea of the current approach comes from the fact that the map, calculated from the models with omitted parts, does not show these parts at full height. In order to increase the height, an attempt can done to fill the non-interpreted region by some scatters and to try to optimise their diffracting power automatically.

The density growing is based on the assumption that an electron density map of resolution around 2-3 Å already has been interpreted everywhere except in a small region with poor density. A fine grid (with the step of 1 Å or less) covers this region, and every node is occupied by a dummy atom. The scattering factors for these atoms can be similar to the ones of hydrogens, and their initial parameters should correspond to a weak density (temperature factors may be 10-40 Å^2 and occupancies are between 0.1-0.5).

As it was shown by Lunin & Urzhumtsev (1984), refinement of dummy models is an important step in the phase improvement. For the density growing, the most important step is the refinement of occupancies of dummy atoms. Usually, a significant part of the dummy atoms does not want to grow, and their occupancies decrease. These dummy atoms (from 50 to 90%, depending on the case) are removed from the model, and the position of other ones is refined without any restraints.

While removing the dummy atoms with very small occupancy changes very little the R- factor, it is important in order to decrease the number of parameters for the following iterations. The usual rule of having a high ratio of Ndata/Nparameters is also applicable in this case. Starting from the occupancy refinement is useful because there is only one parameter per dummy atom at this step, and before position refinement (3 parameters per atom) the number of dummy atoms is already reduced according to the refined occupancy values.

This procedure of refinement of occupancy and atomic positions is automatically repeated varying the low-resolution end. It is important to include all low-resolution data at the initial iterations in order to form the correct shape of the «field» and to remove the dummy atoms from the zones of the bulk solvent. On the contrary, it is useful to finish the iterations with the data of only higher resolution by restricting the low-resolution end to 6-7 Å. Usually, 5-6 iterations are enough, with 20-30 refinement cycles par iteration. Since no geometrical criterion is applied, the procedure is relatively fast.

Structure factor amplitudes calculated from the refined model are used to estimate phase errors in the corresponding calculated phases (Lunin & Skovoroda, 1995). These values are used to prepare the weighted electron density map (Read, 1986; Urzhumtsev et al., 1996). For several experimental data sets the new map showed improved density in the cases of :

a) missed side chain(s) ;
b) poor density at the N-terminal of the main chain ;
c) poor density for an inhibitor in protein-inhibitor complexes.

An analysis of the refined position of dummy atoms did not show any direct way of using them as a guide for the construction of the missing part of the molecule model.

This procedure can also be used instead of simple calculation of the omit-maps (Bhat & Cohen, 1984). The program automatically removes the model atoms from a given region, fills this region with dummy atoms and calculates new structure factors from such mixed model after refinement of the dummy component (which in the case of classical omit-map has zero contribution). Analysis of experimental cases has proven that new maps have an additional information in comparison with the usual omit maps. An intermediate step of the simulated- annealing refinement of the known part of the model (Hodel et al., 1992) before refinement of dummy atoms was also tried. In this case, the new improved map also had more details than the omit one. Retrieval of the density for the well-defined atoms removed from the analysed region can be used as a criterion of the quality of the new map.

The experience showed that «growing» the density (start from small values of occupancy allowing some of them to increase) is more efficient than «suppressing» density (start from large values of occupancy). It is interesting to note that the occupancy of some «grown» dummy atoms of the hydrogen type can be increased to quite large values (2-4).

The same approach is currently being tested for density improvement in the case without an assigned atomic model.

3. Density growing : computer realisation.

The procedure starts from an atomic model and a file of structure factors (FOBS, SIG, TEST values) and produces a map, locally improved in a region of the unit cell. The region is defined by a set of spheres of a prescribed radius around given points. The overlapping parts of spheres contains only one grid corresponding to the sphere «coming first». The procedure consists of 5 steps and is based on X-PLOR (Brünger, 1992). A few parameters at the top of corresponding example files (command files or X-PLOR input files) should be changed to tune the procedure. The main steps are the following.

1. Generation of a dummy-atoms model (program : spherxpl)
   Input :
   - initial atomic model
   - set of points defining the region for improvement (PDB-type lines)
   - file with control data

   Output :
   - atomic model; atoms inside the region are removed (occupancy q = 0)
   - a set of dummy atoms placed in a grid in the defined region
2. Calculate structure factors FPART from atomic model (X-PLOR)
   Input :

- atomic model; atoms inside the region are removed (occupancy q = 0)
- structure file for the atomic model (the same used before for the model refinement)
- structure factors file with FOBS, SIG, TEST
- file with unit cell parameters and symmetries (X-PLOR lines)

Output : - structure factors file in the X-PLOR format with atomic structure factors as FPART

3. Dummy-atom model refinement (X-PLOR)
   Input :
   - model of dummy atoms obtained at step 1
   - file of structure factors calculated at step 2
   - file with unit cell and symmetries

   Output :
   - file of structure factors calculated from a mixed model ("real" and dummy atoms)
4. Phase error estimations (rflexpl, Urzhumtsev et al., 1996)
   Input :
   - file of calculated structure factor at step 3.

   Output :
   - file of structure factors with estimated fom's
5. Map calculation (X-PLOR)
   Input :
   - file of structure factors after the step 4

   Output :
   - a 2Fo-Fc map calculated following Read (1986) with more correctly estimated parameters (Lunin & Skovoroda (1995))

As it was mentioned above, step (1*) of the simulated-annealing refinement (Hodel et al., 1992) of the omit model can be introduced after step (1). This step can be useful in the case of omit-map calculations to check the already built model. Since the omit-model after step (1) has the same list of atoms (omitted atoms are marked by zero occupancy), one can use exactly the same X-PLOR input files for the refinement which were used before.

The programs SPHERXPL and RFLEXPL are written on standard FORTRAN-77 and are available by request from the author. The corresponding e-mail address is sacha@igbmc.u-strasbg.fr.

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